Preserving genome integrity in human cells via DNA double-strand break repair

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ABSTRACT **The efficient maintenance of genome integrity in the face of cellular stress is vital to protect against human diseases such as cancer. DNA replication, chromatin dynamics, cellular signaling, nuclear architecture, cell cycle checkpoints, and other cellular activities contribute to the delicate spatiotemporal control that cells utilize to regulate and maintain genome stability. This perspective will highlight DNA double-strand break (DSB) repair pathways in human cells, how DNA repair failures can lead to human disease, and how PARP inhibitors have emerged as a novel clinical therapy to treat homologous recombination-deficient tumors. We briefly discuss how failures in DNA repair produce a permissive genetic environment in which preneoplastic cells evolve to reach their full tumorigenic potential. Finally, we conclude that an in-depth understanding of DNA DSB repair pathways in human cells will lead to novel therapeutic strategies to treat cancer and potentially other human diseases.**

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INTRODUCTION

Genome integrity is paramount to sustaining the blueprint of life in both somatic and germ cells. Human cells rely on an intricate and highly regulated surveillance system that relentlessly scans the genome for issues that could compromise the integrity of DNA. Environmental and endogenous insults to DNA arise from a myriad of sources including free radicals from the oxygen we breathe, reactive metabolic byproducts, and other biochemical reactions in the cell. While multiple DNA repair pathways strive to correct damage, at times, these repair systems are pushed beyond their limits leading to the accumulation of mutations that result in human diseases such as cancer. DNA double-strand breaks (DSBs) pose a particularly large threat as these lesions lead to cell death if left unrepaired. In this brief review, we will focus on two extensively studied pathways utilized in human cells for the repair of DNA DSBs: nonhomologous end-joining (NHEJ) and homologous recombination (HR). These two DSB repair pathways result in dramatically different outcomes in terms of genome fidelity and may play a significant role in the underlying accumulation of mutations that lead to neoplastic transformation. HR proteins have recently been found to play an important role in DNA replication by protecting stalled forks from degradation and facilitating fork restart by processing lesions that impede the fork. These discoveries have fueled interest in understanding how PARP inhibitors (PARPi) may trap PARP1 on DNA creating a physical blockade to replication and require the action of HR proteins to repair and restart forks. As PARPi selectively kill HR-deficient tumors (e.g., BRCA-deficient tumors), understanding how DNA DSB repair proteins respond to and process DNA damage has important clinical implications.

CANONICAL PATHWAYS FOR REPAIR OF CHROMOSOMAL BREAKS

DNA DSBs are regarded as the most cytotoxic form of DNA damage and occur as a result of normal cellular processes, ionizing radiation (IR), and chemotherapeutics (Limp-Foster and Kelley, 2000; Lees-Miller and Meek, 2003; Lieber, 2010; Kelley, 2012). The cell must repair DSBs in a timely manner; failure to do so can lead to genetic disorders, aging, and cancer. In human cells, DSBs are repaired via two essential pathways linked to the cell cycle (Figure 1A,

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Abbreviations used: ATM, Ataxia Telangiectasia mutated; BLM, Bloom's Syndrome; DDR, DNA damage response; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; DSB, double-strand break; FA, Fanconi Anemia; HDR, homology-directed repair; HR, homologous recombination; IR, ionizing radiation; LIG4, Ligase 4; LOH, loss-of-heterozygosity; NBS1, Nijmegen Breakage Syndrome; NHEJ, nonhomologous end-joining; PARPi, PARP inhibitors; ssDNA, single-stranded DNA; WRN, Werner's Syndrome.

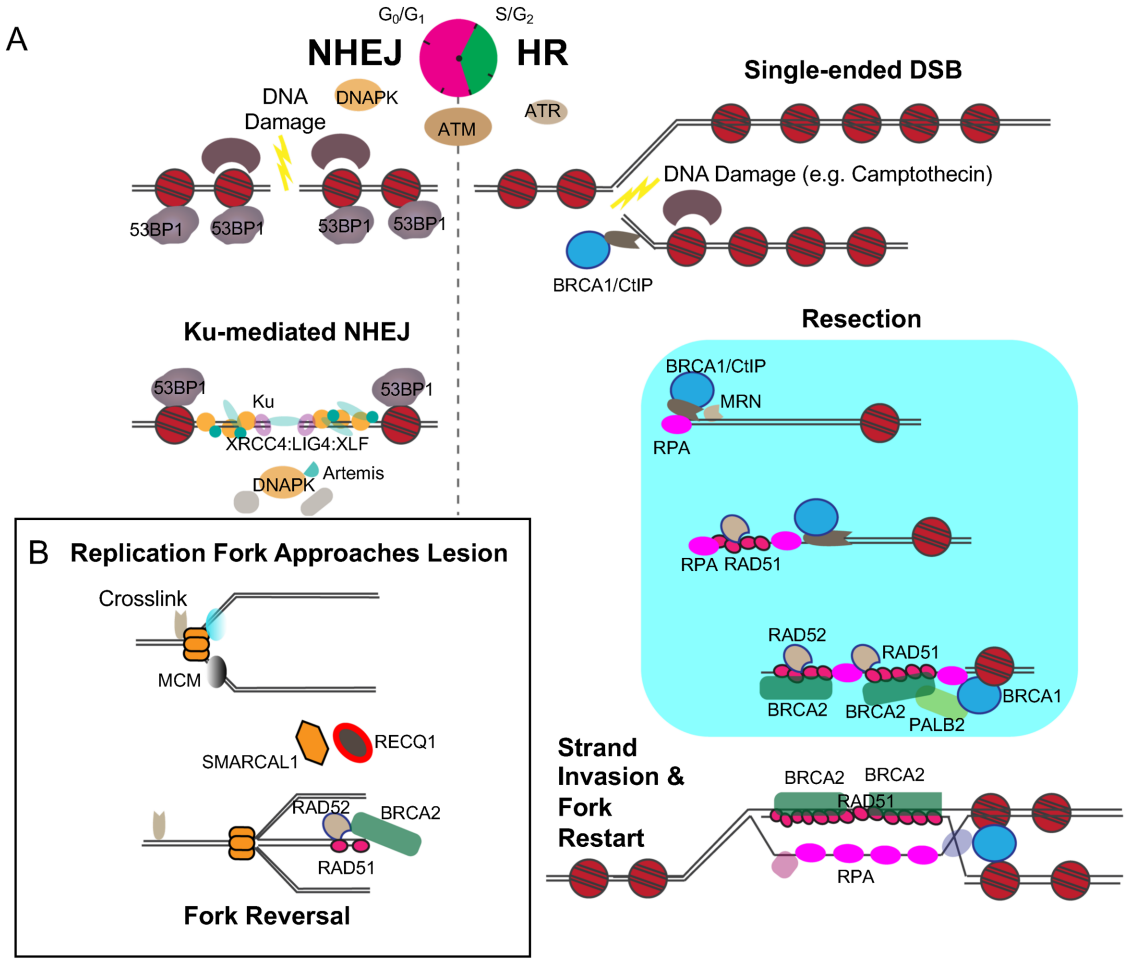

FIGURE 1: Molecular pathways of human DNA DSB repair. (A) DSB repair pathways are strongly regulated by the cell cycle, with NHEJ (left side) active throughout the cell cycle, whereas HDR (right side) acts mainly during replication and is utilized for the repair of damaged replication forks. Each pathway utilizes different repair factors and pathway choice is regulated by diverse requirements including: chromatin environment, site of damage, type of lesion, extent of damage, and proximity to ongoing replication and transcription. All of these factors influence the recruitment, retention, and exclusion of various repair and DDR factors via unknown mechanisms. Following formation of DSBs, DDR is activated by the PI3-like kinases: ATM, DNA-PK, and ATR. **NHEJ (left)** accumulation of 53BP1 at damage sites, binding of Ku, and formation of XLF, XRCC4, and LIG4 filament complexes facilitate end bridging and ligation. Depending on the complexity of the damage, other factors such as DNA-PKcs-Artemis and polymerases may be recruited to allow further processing. **HR (right)** is utilized for repair of damaged/stalled replication forks and involves recruitment of BRCA1 along with a suite of nucleases and associated protein complexes (CtIP, MRE11/RAD50/NBS1, BLM/EXOI, DNA2) to initiate resection. Following resection, ssDNA is bound by RPA. BRCA2 is essential for stabilizing RAD51 filaments and promoting strand invasion and the homology search. BRCA1 remains associated with the damage to enable further recruitment of BRCA2 via PALB2. Following the homology search further steps are required for the resolution of the resulting DNA junctions. (B) Fork remodeling and fork protection in replication stress. Current models propose topological stress (e.g., cross-linked DNA) or a lesion formed ahead of the replication fork will result in reversal of the replication fork mediated by factors such as RECQ1 and SMARCAL1. Following fork reversal, the reversed nascent DNA is likely protected by multiple HDR-associated proteins including BRCA2, RAD51, and RAD52 (note: several DNA repair factors have been omitted from the figure for clarity).

top): NHEJ and HR, or homology-directed repair (HDR) (Lieber, 2010; Frit *et al.*, 2014). Each pathway employs a distinct set of core repair proteins forming functional repair complexes. The NHEJ pathway is the principal (and most rapid) pathway for DSB repair, is active throughout the cell cycle, and involves pairing, processing, and ligation of the broken ends. NHEJ also functions in B- and T-cell immune diversifying pathways by joining DSBs formed during V(D)J and class switch recombination (Lieber, 2010; Ramsden *et al.*, 2010). The HDR pathway is active during DNA replication in S/G_2 phase. A resection step commits the cell to repair by HDR resulting in single-stranded DNA (ssDNA) tails which can then invade the sister chromatid template to initiate the homology search, strand pairing, and exchange of genetic information (Daley and Sung, 2014).

NHEJ

As depicted in Figure 1 (left middle), the core (or minimal) NHEJ complex is composed of the Ku70/Ku80 heterodimer (Ku), DNA Ligase 4 (LIG4)-XRCC4 complex (LX), and the XRCC4 Like factor (XLF). NHEJ can facilitate highly efficient repair of blunt and compatible DNA ends that do not require additional processing steps. However,

the core NHEJ repair complex is modular and can recruit other repair factors to the break if processing is required (Chang *et al.*, 2017). These factors include a nuclease complex composed of Ku, DNA-PKcs (DNA-dependent protein kinase, catalytic subunit) and Artemis, nucleases (Werner's Syndrome [WRN], EXO1), polymerases (Polμ, TdT), and others (Lieber, 2010). Additionally, LIG4 was recently shown to direct processing choice prior to ligation (Conlin *et al.*, 2017; Reid *et al.*, 2017). The initial step of NHEJ repair involves the binding of Ku to DNA ends which acts as a cornerstone for the recruitment and assembly of a functional NHEJ complex (Figure 1, left). Following assembly, a dynamic pairing of the ends forms a synaptic complex, allowing for end alignment and ligation of the broken ends. While two early biochemical studies have suggested a possible role for DNA-PKcs in mediating synapsis (DeFazio *et al.*, 2002; Weterings *et al.*, 2003), numerous other studies have shown that efficient synapsis and joining are predominantly mediated by Ku, XLF, XRCC4, and LIG4 (Ahnesorg *et al.*, 2006; Lu *et al.*, 2007; Wu *et al.*, 2007; Li *et al.*, 2008; Akopiants *et al.*, 2009; Hammel *et al.*, 2010; Andres and Junop, 2011; Hammel *et al.*, 2011; Ropars *et al.*, 2011; Wu *et al.*, 2011; Andres *et al.*, 2012; Roy *et al.*, 2012; Mahaney *et al.*, 2013; Brouwer *et al.*, 2016; Nemoz *et al.*, 2018; Liu *et al.*, 2019; Zhao *et al.*, 2019).

Perhaps surprisingly, we and others have observed that XLF, XRCC4, and LIG4 form multimeric filaments both in vitro and in cells. These extended structures can facilitate pairing, synapsis, and alignment of the broken ends, while partial disruption of these filaments, via abrogated XLF–Ku interaction, results in repair deficit and radiation sensitivity (Ropars *et al.*, 2011; Andres *et al.*, 2012; Mahaney *et al.*, 2013; Reid *et al.*, 2015; Brouwer *et al.*, 2016; Nemoz *et al.*, 2018). It is important to note that despite these observations, we still have a very limited understanding of these filamentous complexes, including their structural, biochemical, and biophysical properties as well as how they form and localize in cells, their specific functional roles, and their interaction with chromatin. Further biochemical and structural studies of these complexes and the assembly and stability of the NHEJ machinery will provide new and improved strategies for targeting DSB repair and therapy resistance.

The kinase activity of the Ataxia Telangiectasia mutated (ATM) protein is crucial for the DNA damage response (DDR). Both ATM and DNA-PKcs phosphorylate several relevant factors associated with DNA damage signaling, yet the repair-specific role of DNA-PKcs is still subject to uncertainty. The previously ascribed cellular role of DNA-PKcs in NHEJ may stem from its functional requirement exemplified in V(D)J recombination. During V(D)J recombination, the DNA-PKcs-Artemis complex facilitates the opening of terminal hairpins at coding ends prior to ligation. However, some studies have demonstrated that DNA-PKcs is dispensable for joining blunt signal ends (Kulesza and Lieber, 1998). Several reports place the role of DNA-PKcs and Artemis at the repair of complex DNA lesions; however, one recent study indicated that depletion of DNA-PKcs had no effect on the efficiency of DSB repair by NHEJ (Liu *et al.*, 2019). These conflicting findings indicate that further studies are required to determine the contribution of DNA-PKcs to the NHEJ repair process. Consequently, the use of DNA-PK inhibitors as a means to block NHEJ repair must be carefully reexamined in both research and clinical applications (Brandsma *et al.*, 2017).

HR AND COMPLEX DNA LESIONS

Experimentally induced DNA DSBs derived from IR, radiomimetic chemotherapeutics, or nuclease-induced DSBs, while readily available in the laboratory setting, may not be relevant to the sources of DNA damage that ultimately result in mutations necessary for neoplastic transformation. Clearly, cancer cells are susceptible to DNA damage. The majority of our clinical efforts (chemotherapeutics, radiation) to eliminate rapidly dividing tumor cells are based on a therapeutic index that can damage the genome of a tumor cell beyond its capacity for repair while sparing normal cells. The majority of DNA DSBs in the human genome are expected to be dealt with by the NHEJ machinery. However, complex lesions resulting from cross-linking agents, topoisomerase inhibitors, PARPi, and other agents that induce topologically complex structures likely rely on HDR for eventual repair. HDR utilizes many more factors than NHEJ including nucleases (e.g., MRE11, EXO1, DNA2), recombination mediators (e.g., BRCA1, BRCA2, PALB2, RAD52), recombinase complexes (e.g., RAD51, RAD51 paralogues, RPA, RAD54), helicases, and resolvases (Delacote and Lopez, 2008; Cerbinskaite *et al.*, 2012). The complete HDR reaction and its regulation are still subject to much uncertainty as it contains many sequential steps and subprocesses that occur over an extended period of time (6–12 h compared with 1 h for NHEJ) (Reid *et al.*, 2015; Whelan *et al.*, 2018), where each of these steps requires the initiation and/or completion of prior steps, and their interruption can lead to diverse repair intermediates and byproducts. The key steps (see illustration in Figure 1, right) of HDR consists of the following: **1**) resection of dsDNA by nucleases, **2**) loading of RAD51 onto RPA-coated ssDNA to form the nucleoprotein filament (mediated by BRCA2), **3**) strand invasion and homology search, and **4**) resolution of the resulting DNA structure (Holliday junctions). While NHEJ provides the most rapid form of repair throughout the cell cycle, during DNA replication, HR and NHEJ are active and can facilitate repair. Since the NHEJ pathway is reactive toward blunt dsDNA ends, whereas the HR pathway requires long ssDNA, it has been postulated that the resection of dsDNA by HR nucleases is a decisive step in pathway choice (Symington and Gautier, 2011; Shao *et al.*, 2012; Sun *et al.*, 2012). Experimental and clinical evidence have confirmed the importance of HDR in complex and replication-associated lesions, as patients and cell lines with HDR defects are exquisitely sensitive to cross-linking agents (e.g., cisplatin, mitomycin C) and PARPi.

DNA REPAIR AT REPLICATION FORKS

Recent studies in the DNA repair field have focused on how DNA lesions can physically impede DNA replication fork progression and the role of HDR proteins in protecting stalled or blocked forks from nucleolytic degradation. The initial idea that replication forks may reverse course after encountering damage was proposed by Hotchkiss and Higgins (Hotchkiss, 1974; Higgins *et al.*, 1976). These reversed forks are colloquially known as "chicken foot" structures due to the three toes representing the leading, lagging, and annealed nascent DNA essentially backing up the replication fork behind the damage (Figure 1B). Fork reversal allows replication to essentially "stall" for time while the DNA repair machinery repairs the damage and prepares the fork to reinitiate replication. Zellweger *et al.* (2015) reported the visual evidence of these structures by electron microscopy, that is, the presence of ssDNA at regressed forks in response to genotoxic agents such as camptothecin or replication stress through hydroxyurea treatment (Zellweger *et al.*, 2015). Further evidence demonstrated that RAD51 was present at reversed forks and may protect regressed arms from nucleolytic degradation (Hashimoto *et al.*, 2010; Schlacher *et al.*, 2011). Improved techniques such as iPOND (isolation of proteins on nascent DNA) and super resolution microscopy have now identified several proteins at stalled forks: SMARCAL1, ZRANB3, HLTF, RECQ1, Bloom's Syndrome (BLM), WRN, FBH1, Fanconi Anemia (FA) proteins, and many others (Betous *et al.*, 2013; Kolinjivadi *et al.*, 2017; Taglialatela *et al.*, 2017; Zadorozhny *et al.*, 2017).

It remains unclear why so many proteins involved at different steps of DNA repair are enriched at stressed replication forks, but undoubtedly cells have a vested interest in paying close attention to molecular events that threaten genomic integrity. BRCA1 and BRCA2 have been found to play key roles at damaged replication forks either in protecting the DNA from nucleolytic degradation and/or providing an HDR-dependent path to fork restart. Considerable uncertainties exist with respect to the properties and mechanisms of fork protection-degradation and reversal processes and whether they are distinct from HDR intermediates at collapsed replication forks. Biochemical and/or superresolution microscopy-based cell biological evidence that BRCA1/2, RAD51, and related factors play a direct role in fork protection/restart remains elusive; however, these studies are eagerly anticipated.

THE BRCA PARADOX: CANCER DRIVER AND ACHILLES' HEEL

Women who inherit a pathogenic germline mutation in BRCA2 face a serious lifetime risk of breast and ovarian cancer, whereas men face increased risk of pancreatic, prostate, and also breast cancer. BRCA2 is involved in the HDR pathway of DNA DSB repair first discovered through its interaction with RAD51, a central player in HR (Wong *et al.*, 1997). The RAD51 protein forms a nucleoprotein filament on ssDNA and initiates a search for homology presumably in the sister chromatid (Benson *et al.*, 1994). BRCA2 acts as a "mediator" to both bind and load RAD51 onto resected DNA DSBs (Jensen *et al.*, 2010; Chatterjee *et al.*, 2016). In the absence of BRCA2, RAD51 functions are compromised and HDR repair is short circuited. Loss-of-function mutations in BRCA1/2 and other HDR pathway genes (e.g., PALB2, RAD51 paralogues) can be leveraged therapeutically as they confer sensitivity to cross-linkers, platinum agents, and PARPi.

Despite BRCA2 mutation carriers possessing one wild-type copy of the allele, tumors from most patients display loss-of-heterozygosity (LOH) and are null for BRCA2 function. In some patients with germline mutations, the wild-type BRCA2 allele is retained in the tumor (Maxwell *et al.*, 2017), and in this scenario, perhaps haploinsufficiency alone may favor tumor formation. When LOH has occurred in the tumor, the molecular ordering of events that underpin loss of the wild-type allele remains largely unknown and the genetic environment permissive for loss of BRCA2 function is an active area of investigation. This is an important point as BRCA2 knockout in mice is early embryonic lethal and knockout/knockdown in human cell culture models is usually associated with inviability (Feng and Jasin, 2017). This raises a somewhat paradoxical situation whereby BRCA2 is essential for cellular growth and viability, and yet, loss of BRCA2 can drive tumorigenesis in a specific genetic context. Furthermore, BRCA-deficient tumors are compromised for HDR repair providing a unique opportunity for therapeutic intervention with PARPi. Recent genetic studies have pointed toward a role for BRCA2 in stabilizing RAD51 at stalled replication forks and have postulated that fork protection is critical in response to PARPi (Schlacher *et al.*, 2011; Ray Chaudhuri *et al.*, 2016). Surprisingly, the HDR functions of BRCA2 and RAD51 appear dispensable in the replication fork protection model. Adding further to the controversy, a recent study demonstrated treatment of BRCA mutant cells results in increased replication fork speed rather than reduced velocity or fork stalling/collapse (Maya-Mendoza *et al.*, 2018). Clearly, more work needs to be done to parse out mechanistically why BRCA proteins, and more generally the HDR pathway, is required to manage PARPi-mediated damage. To date, very few studies have utilized reconstituted biochemical assays using purified proteins and defined DNA substrates to address the mechanistic details of PARPi-dependent DNA lesions. We propose that biochemically defined systems, single molecule analysis, and superresolution visualization of these repair proteins at PARPi-induced lesions will greatly facilitate our understanding of how these clinically important molecules specifically kill HDR-deficient cells.

PARPI: THE DNA REPAIR BLOCKBUSTER DRUG

The discovery that BRCA mutant cells are selectively killed by PARPi (Bryant *et al.*, 2005; Farmer *et al.*, 2005) opened up a completely new therapeutic avenue for patients carrying germline BRCA mutations. Lynparza (otherwise known as Olaparib) was the first FDA-approved PARPi for the treatment of patients with BRCA-deficient ovarian cancer (Kim *et al.*, 2015) and despite vigorous research over the past 15 years into the underlying mechanisms, we still do not know why HDR-deficient cells are selectively targeted by PARPi and how HDR-competent cells are spared. Multiple PARPi formulations have emerged from pharmaceutical companies in various stages of development from preclinical testing to FDA approval; many questions remain surrounding issues of potency and optimal treatment regimens.

PARP1 is the founding family member of a class of DNA damage sensors involved in attaching poly (ADP-ribose) PAR chains to itself (a term denoted PARylation) and target proteins at DNA lesions (Lord and Ashworth, 2017). Knockout of PARP1 and a second family member, PARP2, is individually viable in a mouse knockout; however, the double knockout *PARP1*$^{-/-}$*PARP2*$^{-/-}$ is embryonic lethal (Menissier de Murcia *et al.*, 2003). It is thought that PARP1 signals the presence of DNA damage, recruits various DNA repair proteins to the lesion, and eventually disengages from the DNA (through autoPARylation of itself) once appropriate DNA repair enzymes have been recruited (Lindahl *et al.*, 1995; Pommier *et al.*, 2016; Lord and Ashworth, 2017). The protein PARG (poly (ADP-ribose) glycohydrolase) acts as a negative regulator of PARP1 removing PARylation marks and thus, shutting down the DDR (Barkauskaite *et al.*, 2015). The majority of PARP research has focused on PARP1, but interestingly, all clinical PARPi can inhibit both PARP1 and PARP2. The concept of "PARP trapping" whereby the PARP1 protein becomes stuck in front of a progressing replication fork has recently gained favor as the proposed mechanism of action (Murai *et al.*, 2012, 2014). Some models suggest that prevention of catalytic PARylation underlies the synthetic lethal effects of PARPi; however, inhibition of PARylation does not seem to track with the cytotoxicity of different PARPi (Murai *et al.*, 2012). The physical impediment of trapped PARP1 appearing as a DNA lesion may explain why removal or bypass can only be achieved using an HDR-dependent mechanism hence explaining the severe sensitivity of BRCA and other HDR-deficient cells. Whether HDR-deficient cells exposed to PARPi die by apoptosis or a mitotic catastrophe-like death from unreplicated DNA remaining following mitosis is still an open question.

GENOME INTEGRITY AND DNA REPAIR

Genome instability and dysfunctional DNA repair are linked to the etiology of severe human syndromes including developmental, immunological, and neurological disorders and cancer (McKinnon and Caldecott, 2007; Rass *et al.*, 2007; Wilson *et al.*, 2008; Lieber, 2010; Wu and Brosh, 2010; Wysham *et al.*, 2012; Canugovi *et al.*, 2013; Suhasini and Brosh, 2013; Vindigni and Gonzalo, 2013; Chang *et al.*, 2017). Some examples of inherited genetic syndromes resulting from dysfunctional DNA damage signaling or repair include: ATM, Nijmegen Breakage Syndrome (NBS1), BLM, WRN, FA, Hereditary Nonpolyposis Colorectal Cancer (MLH1, MSH2), and hereditary

breast and ovarian cancer (BRCA1, BRCA2, PALB2), among others. Genomic instability is likely the driving force that fuels neoplastic potential in the opposition of cellular factors aimed at suppressing cellular transformation. This hypothesis was elegantly proposed as the "mutator phenotype" by Larry Loeb in 1974 (Loeb *et al.*, 1974). Accumulation of DNA damage followed by less than perfect repair offers a rich source of mutational events in critical gatekeeper pathways including tumor suppressor genes and oncogenes. In fact, these mutational events driven by DNA damage are likely the lynchpin mechanism overcoming the many barriers to transformation such as cellular proliferative capacity, angiogenesis, altered metabolic pathways, and others (Hanahan and Weinberg, 2011). Achieving an ideal balance of genetic malleability to foster tumor growth is not an easy feat and probably explains why cancer incidence is not more prevalent in the human population. Of course, age is strongly correlated with increasing cancer rates reflecting the accumulation of DNA damage over time. Continued exploration of DNA repair networks will provide an unprecedented opportunity to reveal the underlying biology of malignant transformation. Elucidation of these networks will guide the development of novel therapeutic strategies with enhanced selectivity and reduced toxicity to cancer patients.

FINAL THOUGHTS AND CONCLUSION

Despite much progress in the past few decades, we still face substantial gaps in our knowledge surrounding DNA repair mechanisms in human cells, how malfunctions lead to disease, and how DNA repair deficits can be further utilized in targeted therapy. While the first targeted DNA repair therapy—PARPi—has enjoyed its share of clinical successes for certain tumor types, resistance and relapse remain an ongoing problem. Many clever genetic and proteomic screens conducted in BRCA-mutant cell lines have identified loss-of-function genes (53BP1, REV7, Shieldin complex) that confer resistance to PARPi (Jaspers *et al.*, 2013; Xu *et al.*, 2015; Gupta *et al.*, 2018; Noordermeer *et al.*, 2018); however, clinical identification of mutations in these specific genes in actual patent tumors has remained sparse. Surprisingly, studies from breast and ovarian patient tumors resistant to cisplatin and PARPi revealed secondary reversion mutations in BRCA1 and BRCA2 back to a "wild type-like" functional state and are a potential source of bona fide resistance (Edwards *et al.*, 2008; Sakai *et al.*, 2008; Christie *et al.*, 2017). Combination therapies including WEE1, ATRi, and CHK1/2 inhibitors that impair cell cycle checkpoint regulation are being tested in clinical trials with the hope they will potentiate PARPi toxicity. In order for physicians to select patients ideally suited for PARPi treatment and choose optimal combination therapies to combat resistance mechanisms, a detailed understanding of the underlying mechanism of DNA repair and PARPi is greatly needed. Another barrier hindering the progress of new therapeutic interventions is the lack of strategies for targeting NHEJ—the major DSB repair pathway. Further research focusing on the specific roles of the various structural proteins of the NHEJ machinery will provide much needed alternative approaches for targeting DNA repair (McFadden *et al.*, 2014). Insights derived from concentrated efforts using basic experimental approaches, including biochemical, structural, and cell biology assays, along with advanced single-molecule studies have great potential to fill in these important missing details.

REFERENCES

Ahnesorg P, Smith P, Jackson SP (2006). XLF interacts with the XRCC4-DNA ligase IV complex to promote DNA nonhomologous end-joining. Cell 124, 301–313.

Akopiants K, Zhou RZ, Mohapatra S, Valerie K, Lees-Miller SP, Lee KJ, Chen DJ, Revy P, de Villartay JP, Povirk LF (2009). Requirement for XLF/Cernunnos in alignment-based gap filling by DNA polymerases lambda and mu for nonhomologous end joining in human whole-cell extracts. Nucleic Acids Res 37, 4055–4062.

Andres SN, Junop MS (2011). Crystallization and preliminary X-ray diffraction analysis of the human XRCC4-XLF complex. Acta Crystallogr Sect F Struct Biol Cryst Commun 67, 1399–1402.

Andres SN, Vergnes A, Ristic D, Wyman C, Modesti M, Junop M (2012). A human XRCC4-XLF complex bridges DNA. Nucleic Acids Res 40, 1868–1878.

Barkauskaite E, Jankevicius G, Ahel I (2015). Structures and mechanisms of enzymes employed in the synthesis and degradation of PARP-dependent protein ADP-ribosylation. Mol Cell 58, 935–946.

Benson FE, Stasiak A, West SC (1994). Purification and characterization of the human Rad51 protein, an analogue of E. coli RecA. EMBO J 13, 5764–5771.

Betous R, Couch FB, Mason AC, Eichman BF, Manosas M, Cortez D (2013). Substrate-selective repair and restart of replication forks by DNA translocases. Cell Rep 3, 1958–1969.

Brandsma I, Fleuren EDG, Williamson CT, Lord CJ (2017). Directing the use of DDR kinase inhibitors in cancer treatment. Expert Opin Investig Drugs 26, 1341–1355.

Brouwer I, Sitters G, Candelli A, Heerema SJ, Heller I, de Melo AJ, Zhang H, Normanno D, Modesti M, Peterman EJ, *et al.* (2016). Sliding sleeves of XRCC4-XLF bridge DNA and connect fragments of broken DNA. Nature 535, 566–569.

Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, Kyle S, Meuth M, Curtin NJ, Helleday T, *et al.* (2005). Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature 434, 913–917.

Canugovi C, Misiak M, Ferrarelli LK, Croteau DL, Bohr VA (2013). The role of DNA repair in brain related disease pathology. DNA Repair 12, 578–587.

Cerbinskaite A, Mukhopadhyay A, Plummer ER, Curtin NJ, Edmondson RJ (2012). Defective homologous recombination in human cancers. Cancer Treat Rev 38, 89–100.

Chang HHY, Pannunzio NR, Adachi N, Lieber MR (2017). Non-homologous DNA end joining and alternative pathways to double-strand break repair. Nat Rev Mol Cell Biol 18, 495–506.

Chatterjee G, Jimenez-Sainz J, Presti T, Nguyen T, Jensen RB (2016). Distinct binding of BRCA2 BRC repeats to RAD51 generates differential DNA damage sensitivity. Nucleic Acids Res 44, 5256–5270.

Christie EL, Fereday S, Doig K, Pattnaik S, Dawson SJ, Bowtell DDL (2017). Reversion of BRCA1/2 germline mutations detected in circulating tumor DNA From patients with high-grade serous ovarian cancer. J Clin Oncol 35, 1274–1280.

Conlin MP, Reid DA, Small GW, Chang HH, Watanabe G, Lieber MR, Ramsden DA, Rothenberg E (2017). DNA ligase IV guides end-processing choice during nonhomologous end joining. Cell Rep 20, 2810–2819.

Daley JM, Sung P (2014). 53BP1, BRCA1, and the choice between recombination and end joining at DNA double-strand breaks. Mol Cell Biol 34, 1380–1388.

DeFazio LG, Stansel RM, Griffith JD, Chu G (2002). Synapsis of DNA ends by DNA-dependent protein kinase. EMBO J 21, 3192–3200.

Delacote F, Lopez BS (2008). Importance of the cell cycle phase for the choice of the appropriate DSB repair pathway, for genome stability maintenance: the trans-S double-strand break repair model. Cell Cycle 7, 33–38.

Edwards SL, Brough R, Lord CJ, Natrajan R, Vatcheva R, Levine DA, Boyd J, Reis-Filho JS, Ashworth A (2008). Resistance to therapy caused by intragenic deletion in BRCA2. Nature 451, 1111–1115.

Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, Santarosa M, Dillon KJ, Hickson I, Knights C, *et al.* (2005). Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature 434, 917–921.

Feng W, Jasin M (2017). BRCA2 suppresses replication stress-induced mitotic and G1 abnormalities through homologous recombination. Nat Commun 8, 525.

Frit P, Barboule N, Yuan Y, Gomez D, Calsou P (2014). Alternative end-joining pathway(s): bricolage at DNA breaks. DNA repair 17, 81–97.

Gupta R, Somyajit K, Narita T, Maskey E, Stanlie A, Kremer M, Typas D, Lammers M, Mailand N, Nussenzweig A, *et al.* (2018). DNA repair network analysis reveals Shieldin as a key regulator of NHEJ and PARP inhibitor sensitivity. Cell 173, 972–988.e23.

Hammel M, Rey M, Yu Y, Mani RS, Classen S, Liu M, Pique ME, Fang S, Mahaney BL, Weinfeld M, *et al.* (2011). XRCC4 protein interactions with XRCC4-like factor (XLF) create an extended grooved scaffold for DNA ligation and double strand break repair. J Biol Chem 286, 32638–32650.
Hammel M, Yu Y, Fang S, Lees-Miller SP, Tainer JA (2010). XLF regulates filament architecture of the XRCC4.ligase IV complex. Structure 18, 1431–1442.
Hanahan D, Weinberg RA (2011). Hallmarks of cancer: the next generation. Cell 144, 646–674.
Hashimoto Y, Ray Chaudhuri A, Lopes M, Costanzo V (2010). Rad51 protects nascent DNA from Mre11-dependent degradation and promotes continuous DNA synthesis. Nat Struct Mol Biol 17, 1305–1311.
Higgins NP, Kato K, Strauss B (1976). A model for replication repair in mammalian cells. J Mol Biol 101, 417–425.
Hotchkiss RD (1974). Models of genetic recombination. Annu Rev Microbiol 28, 445–468.
Jaspers JE, Kersbergen A, Boon U, Sol W, van Deemter L, Zander SA, Drost R, Wientjens E, Ji J, Aly A, *et al.* (2013). Loss of 53BP1 causes PARP inhibitor resistance in Brca1-mutated mouse mammary tumors. Cancer Discov 3, 68–81.
Jensen RB, Carreira A, Kowalczykowski SC (2010). Purified human BRCA2 stimulates RAD51-mediated recombination. Nature 467, 678–683.
Kelley MR (ed) (2012). DNA Repair in Cancer Therapy: Molecular Targets and Clinical Applications, London; Waltham, MA: Elsevier/Academic Press, xiii.
Kim G, Ison G, McKee AE, Zhang H, Tang S, Gwise T, Sridhara R, Lee E, Tzou A, Philip R, *et al.* (2015). FDA approval summary: olaparib monotherapy in patients with deleterious germline BRCA-mutated advanced ovarian cancer treated with three or more lines of chemotherapy. Clin Cancer Res 21, 4257–4261.
Kolinjivadi AM, Sannino V, De Antoni A, Zadorozhny K, Kilkenny M, Techer H, Baldi G, Shen R, Ciccia A, Pellegrini L, *et al.* (2017). Smarcal1-mediated fork reversal triggers Mre11-dependent degradation of nascent DNA in the absence of Brca2 and stable Rad51 nucleofilaments. Mol Cell 67, 867–881.e7.
Kulesza P, Lieber MR (1998). DNA-PK is essential only for coding joint formation in V(D)J recombination. Nucleic Acids Res 26, 3944–3948.
Lees-Miller SP, Meek K (2003). Repair of DNA double strand breaks by nonhomologous end joining. Biochimie 85, 1161–1173.
Li Y, Chirgadze DY, Bolanos-Garcia VM, Sibanda BL, Davies OR, Ahnesorg P, Jackson SP, Blundell TL (2008). Crystal structure of human XLF/Cernunnos reveals unexpected differences from XRCC4 with implications for NHEJ. EMBO J 27, 290–300.
Lieber MR (2010). The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. Annu Rev Biochem 79, 181–211.
Limp-Foster M, Kelley MR (2000). DNA repair and gene therapy: implications for translational uses. Environ Mol Mutagen 35, 71–81.
Lindahl T, Satoh MS, Poirier GG, Klungland A (1995). Post-translational modification of poly(ADP-ribose) polymerase induced by DNA strand breaks. Trends Biochem Sci 20, 405–411.
Liu Y, Efimova EV, Ramamurthy A, Kron SJ (2019). Repair-independent functions of DNA-PKcs protect irradiated cells from mitotic slippage and accelerated senescence. J Cell Sci 132.
Loeb LA, Springgate CF, Battula N (1974). Errors in DNA replication as a basis of malignant changes. Cancer Res 34, 2311–2321.
Lord CJ, Ashworth A (2017). PARP inhibitors: synthetic lethality in the clinic. Science 355, 1152–1158.
Lu H, Pannicke U, Schwarz K, Lieber MR (2007). Length-dependent binding of human XLF to DNA and stimulation of XRCC4.DNA ligase IV activity. J Biol Chem 282, 11155–11162.
Mahaney BL, Hammel M, Meek K, Tainer JA, Lees-Miller SP (2013). XRCC4 and XLF form long helical protein filaments suitable for DNA end protection and alignment to facilitate DNA double strand break repair. Biochem Cell Biol 91, 31–41.
Maxwell KN, Wubbenhorst B, Wenz BM, De Sloover D, Pluta J, Emery L, Barrett A, Kraya AA, Anastopoulos IN, Yu S, *et al.* (2017). BRCA locus-specific loss of heterozygosity in germline BRCA1 and BRCA2 carriers. Nat Commun 8, 319.
Maya-Mendoza A, Moudry P, Merchut-Maya JM, Lee M, Strauss R, Bartek J (2018). High speed of fork progression induces DNA replication stress and genomic instability. Nature 559, 279–284.
McFadden MJ, Lee WK, Brennan JD, Junop MS (2014). Delineation of key XRCC4/Ligase IV interfaces for targeted disruption of non-homologous end joining DNA repair. Proteins 82, 187–194.
McKinnon PJ, Caldecott KW (2007). DNA strand break repair and human genetic disease. Annu Rev Genomics Hum Genet 8, 37–55.
Menissier de Murcia J, Ricoul M, Tartier L, Niedergang C, Huber A, Dantzer F, Schreiber V, Ame JC, Dierich A, LeMeur M, *et al.* (2003). Functional interaction between PARP-1 and PARP-2 in chromosome stability and embryonic development in mouse. EMBO J 22, 2255–2263.
Murai J, Huang SY, Das BB, Renaud A, Zhang Y, Doroshow JH, Ji J, Takeda S, Pommier Y (2012). Trapping of PARP1 and PARP2 by clinical PARP inhibitors. Cancer Res 72, 5588–5599.
Murai J, Huang SY, Renaud A, Zhang Y, Ji J, Takeda S, Morris J, Teicher B, Doroshow JH, Pommier Y, *et al.* (2014). Stereospecific PARP trapping by BMN 673 and comparison with olaparib and rucaparib. Mol Cancer Ther 13, 433–443.
Nemoz C, Ropars V, Frit P, Gontier A, Drevet P, Yu J, Guerois R, Pitois A, Comte A, Delteil C, *et al.* (2018). XLF and APLF bind Ku80 at two remote sites to ensure DNA repair by non-homologous end joining. Nat Struct Mol Biol 25, 971–980.
Noordermeer SM, Adam S, Setiaputra D, Barazas M, Pettitt SJ, Ling AK, Olivieri M, Alvarez-Quilon A, Moatti N, Zimmermann M, *et al.* (2018). The shieldin complex mediates 53BP1-dependent DNA repair. Nature 560, 117–121.
Pommier Y, O'Connor MJ, de Bono J (2016). Laying a trap to kill cancer cells: PARP inhibitors and their mechanisms of action. Sci Transl Med 8, 362ps17.
Ramsden DA, Weed BD, Reddy YV (2010). V(D)J recombination: born to be wild. Semin Cancer Biol 20, 254–260.
Rass U, Ahel I, West SC (2007). Defective DNA repair and neurodegenerative disease. Cell 130, 991–1004.
Ray Chaudhuri A, Callen E, Ding X, Gogola E, Duarte AA, Lee JE, Wong N, Lafarga V, Calvo JA, Panzarino NJ, *et al.* (2016). Replication fork stability confers chemoresistance in BRCA-deficient cells. Nature 535, 382–387.
Reid DA, Conlin MP, Yin Y, Chang HH, Watanabe G, Lieber MR, Ramsden DA, Rothenberg E (2017). Bridging of double-stranded breaks by the nonhomologous end-joining ligation complex is modulated by DNA end chemistry. Nucleic Acids Res 45, 1872–1878.
Reid DA, Keegan S, Leo-Macias A, Watanabe G, Strande NT, Chang HH, Oksuz BA, Fenyo D, Lieber MR, Ramsden DA, *et al.* (2015). Organization and dynamics of the nonhomologous end-joining machinery during DNA double-strand break repair. Proc Natl Acad Sci USA 112, E2575–E2584.
Ropars V, Drevet P, Legrand P, Baconnais S, Amram J, Faure G, Marquez JA, Pietrement O, Guerois R, Callebaut I, *et al.* (2011). Structural characterization of filaments formed by human Xrcc4-Cernunnos/XLF complex involved in nonhomologous DNA end-joining. Proc Natl Acad Sci USA 108, 12663–12668.
Roy S, Andres SN, Vergnes A, Neal JA, Xu Y, Yu Y, Lees-Miller SP, Junop M, Modesti M, Meek K (2012). XRCC4's interaction with XLF is required for coding (but not signal) end joining. Nucleic Acids Res 40, 1684–1694.
Sakai W, Swisher EM, Karlan BY, Agarwal MK, Higgins J, Friedman C, Villegas E, Jacquemont C, Farrugia DJ, Couch FJ, *et al.* (2008). Secondary mutations as a mechanism of cisplatin resistance in BRCA2-mutated cancers. Nature 451, 1116–1120.
Schlacher K, Christ N, Siaud N, Egashira A, Wu H, Jasin M (2011). Double-strand break repair-independent role for BRCA2 in blocking stalled replication fork degradation by MRE11. Cell 145, 529–542.
Shao Z, Davis AJ, Fattah KR, So S, Sun J, Lee KJ, Harrison L, Yang J, Chen DJ (2012). Persistently bound Ku at DNA ends attenuates DNA end resection and homologous recombination. DNA Repair 11, 310–316.
Suhasini AN, Brosh RM Jr (2013). Disease-causing missense mutations in human DNA helicase disorders. Mutat Res 752, 138–152.
Sun J, Lee KJ, Davis AJ, Chen DJ (2012). Human Ku70/80 protein blocks exonuclease 1-mediated DNA resection in the presence of human Mre11 or Mre11/Rad50 protein complex. J Biol Chem 287, 4936–4945.
Symington LS, Gautier J (2011). Double-strand break end resection and repair pathway choice. Annu Rev Genet 45, 247–271.
Taglialatela A, Alvarez S, Leuzzi G, Sannino V, Ranjha L, Huang JW, Madubata C, Anand R, Levy B, Rabadan R, *et al.* (2017). Restoration of replication fork stability in BRCA1- and BRCA2-deficient cells by inactivation of SNF2-family fork remodelers. Mol Cell 68, 414–430.e8.
Vindigni A, Gonzalo S (2013). The two faces of DNA repair: disease and therapy. Mo Med 110, 314–319.
Weterings E, Verkaik NS, Bruggenwirth HT, Hoeijmakers JH, van Gent DC (2003). The role of DNA dependent protein kinase in synapsis of DNA ends. Nucleic Acids Res 31, 7238–7246.

Whelan DR, Lee WTC, Yin Y, Ofri DM, Bermudez-Hernandez K, Keegan S, Fenyo D, Rothenberg E (2018). Spatiotemporal dynamics of homologous recombination repair at single collapsed replication forks. Nat Commun 9, 3882.
Wilson DM 3rd, Bohr VA, McKinnon PJ (2008). DNA damage, DNA repair, ageing and age-related disease. Mech Ageing Dev 129, 349–352.
Wong AK, Pero R, Ormonde PA, Tavtigian SV, Bartel PL (1997). RAD51 interacts with the evolutionarily conserved BRC motifs in the human breast cancer susceptibility gene brca2. J Biol Chem 272, 31941–31944.
Wu Y, Brosh RM Jr (2010). G-quadruplex nucleic acids and human disease. FEBS J 277, 3470–3488.
Wu PY, Frit P, Malivert L, Revy P, Biard D, Salles B, Calsou P (2007). Interplay between Cernunnos-XLF and nonhomologous end-joining proteins at DNA ends in the cell. J Biol Chem 282, 31937–31943.
Wu Q, Ochi T, Matak-Vinkovic D, Robinson CV, Chirgadze DY, Blundell TL (2011). Non-homologous end-joining partners in a helical dance: structural studies of XLF-XRCC4 interactions. Biochem Soc Trans 39, 1387–1392, suppl 2 p following 92.
Wysham WZ, Mhawech-Fauceglia P, Li H, Hays L, Syriac S, Skrepnik T, Wright J, Pande N, Hoatlin M, Pejovic T (2012). BRCAness profile of sporadic ovarian cancer predicts disease recurrence. PLoS One 7, e30042.
Xu G, Chapman JR, Brandsma I, Yuan J, Mistrik M, Bouwman P, Bartkova J, Gogola E, Warmerdam D, Barazas M, *et al.* (2015). REV7 counteracts DNA double-strand break resection and affects PARP inhibition. Nature 521, 541–544.
Zadorozhny K, Sannino V, Belan O, Mlcouskova J, Spirek M, Costanzo V, Krejci L (2017). Fanconi-anemia-associated mutations destabilize RAD51 filaments and impair replication fork protection. Cell Rep 21, 333–340.
Zellweger R, Dalcher D, Mutreja K, Berti M, Schmid JA, Herrador R, Vindigni A, Lopes M (2015). Rad51-mediated replication fork reversal is a global response to genotoxic treatments in human cells. J Cell Biol 208, 563–579.
Zhao B, Watanabe G, Morten MJ, Reid DA, Rothenberg E, Lieber MR (2019). The essential elements for the noncovalent association of two DNA ends during NHEJ synapsis. Nat Commun 10, 3588.